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# Tissue Swainsonine Clearance in Sheep Chronically Poisoned with Locoweed<sup>1</sup> (*Oxytropis sericea*)

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**ABSTRACT:** Locoweed poisoning is seen throughout the world and annually costs the livestock industry millions of dollars. Swainsonine inhibits lysosomal  $\alpha$ -mannosidase and Golgi mannosidase II. Poisoned animals are lethargic, anorexic, emaciated, and have neurologic signs that range from subtle apprehension to seizures. Swainsonine is water-soluble, rapidly absorbed, and likely to be widely distributed in the tissues of poisoned animals. The purpose of this study was to quantify swainsonine in tissues of locoweed-poisoned sheep and determine the rate of swainsonine clearance from animal tissues. Twenty-four crossbred wethers were gavaged with ground *Oxytropis sericea* to obtain swainsonine doses of 1 mg swainsonine·kg<sup>-1</sup> BW·d<sup>-1</sup> for 30 d. After dosing, the sheep were killed on d 0, 1, 2, 3, 4, 6, 14, 30, 60, and 160. Animal weights and feed consumption

were monitored. Serum was collected during dosing and withdrawal periods, and tissues were collected at necropsy. Serum swainsonine concentrations were determined using an  $\alpha$ -mannosidase inhibition assay. Swainsonine concentrations in skeletal muscle, heart, brain, and serum were similar at approximately 250 ng/g. Clearance from these tissues was also similar, with half-lives ( $T_{1/2}$ ) of less than 20 h. Swainsonine at more than 2,000 ng/g, was detected in the liver, spleen, kidney, and pancreas. Clearance from liver, kidney, and pancreas was about  $T_{1/2}$  60 h. These findings imply that poisoned sheep have significant tissue swainsonine concentrations and animals exposed to locoweed should be withheld from slaughter for at least 25 d (10  $T_{1/2}$ ) to ensure that the locoweed toxin has cleared from animal tissues and products.

Key Words: Poisonous Weeds, *Oxytropis*, Clearance, Toxicity

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## Introduction

Locoweed poisoning or “locoism” occurs when animals graze for several weeks certain plants of the *Astragalus* or *Oxytropis* genera. Clinical signs of poisoning may be severe or nearly undetectable and include depression, proprioceptive deficits, tremors, nervousness (especially when stressed), dull hair coat, emaciation, decreased libido, infertility, abortion, hydrops amnii, cardiovascular disease, seizures, and occasionally death. Poisoning results in microscopic neuro-visceral vacuolar degeneration in many tissues, but these changes are not grossly visible and they may be undetected when animals are sent to slaughter.

Bowen et al. (1993) previously described the initial distribution and clearance rates of a single intravenous swainsonine injection in mice. Because locoism in livestock is a chronic, insidious disease that takes several weeks to months to develop, swainsonine toxicokinetics in these animals is likely to be much different from that seen in an animal given a single injection. Because it is also possible that animal products or tissues could have swainsonine contamination, information on swainsonine clearance and metabolism in livestock is needed. The purpose of this study was to document swainsonine concentration in tissues of livestock that have been chronically dosed with locoweed and determine the clearance of swainsonine from these animals.

## Materials and Methods

*Oxytropis sericea* from Clayton, Union County, New Mexico was collected, air dried, and ground to pass through a 4-mm screen. The ground plant was mixed and representative samples were collected and analyzed for swainsonine using capillary gas chromatog-

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raphy (Molyneux et al., 1989). This ground plant was dosed twice a day as a slurry in 500 mL of warm water via gavage to 24 mixed-breed wethers (body weight  $\pm$  SD  $41.5 \pm 3.1$  kg) to obtain a swainsonine dose of  $1 \text{ mg swainsonine} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 30 d. Eight additional wethers were gavaged with similar amounts of ground alfalfa as controls. All wethers were individually penned, allowed ad libitum access to water, and consumption of pelleted alfalfa feed was monitored. Through the dosing period, the animals were weighed weekly, feed intake was monitored, and serum was collected for biochemical and swainsonine analysis. At the conclusion of the dosing period, the locoweed-dosed animals were randomly assigned to 10 groups that were killed and necropsied on d 0 (three animals), 1 (three animals), 2 (three animals), 3 (three animals), 4 (three animals), 6 (two animals), 14 (two animals), 30 (two animals), 60 (two animals), and 160 (one animal) relative to the last dose of swainsonine. Control wethers were killed and necropsied on d 0. At necropsy tissues were collected, frozen, and stored at  $-20^{\circ}\text{C}$ , and later analyzed for swainsonine concentrations using previously reported techniques (Stegelmeier et al., 1995a,b). Frozen tissues from control wethers were used for controls. Swainsonine-spiked replicates of these samples were used to develop the standard curve and the swainsonine concentrations in these standards were verified using capillary gas chromatography (Molyneux et al., 1989).

A small pilot study was performed to verify that gavaging ground plant into the rumen of sheep is comparable to locoweed ingestion. Sera were collected every 2 h for 24 h from two groups of five wethers (five from this study and five from a separate study in which the wethers were fed a 10% locoweed [*Oxytropis sericea*]/alfalfa pellet). The sera were analyzed for swainsonine as referenced above. Serum swainsonine concentrations (not shown) were similar for both methods of dosing ( $P = .9$ ) and differences between animals and throughout the day were similar in animals gavaged with locoweed and others eating locoweed pellets ( $P = .8$ ).

Sequential data were analyzed by analysis of variance using a generalized linear model for repeated measures (SAS, 1985). Clearance rates and tissue swainsonine concentrations were analyzed using an analysis of variance (Proc GLM, SAS, 1985). Where significant differences occurred, the means were separated using Duncan's multiple range test (SAS, 1985). Clearance rates were described using linear regression (Proc REG, SAS, 1985).

## Results

All the wethers dosed with locoweed began to show subtle signs of poisoning after 15 d of dosing. Early changes included reluctance to stand, low head

carriage, and inappetence. Later some animals had more severe changes, exhibiting prominent muscular tremors, muscular weakness, and proprioceptive deficits. These clinical changes seemed to become more severe for 2 to 3 d after discontinuing locoweed dosing, and within 5 to 7 d no clinical signs were detectable in any of the sheep. For the first 2 wk of dosing, the wethers continued to gain weight at predosing rates; however, after about 3 wk of locoweed poisoning, the weight gains were significantly less ( $P = .05$ ) than previous rates (Figure 1). These animals refused up to 50% of their ration. Within 7 d after discontinuing locoweed dosing, weight gains and dietary intake returned to rates similar to those seen before dosing (Figure 1). Control wethers were clinically normal and consumed their diets throughout the study (data not shown).

In poisoned animals, swainsonine was detected in all tissues analyzed. Liver, spleen, kidney, and pancreas had the highest concentrations. Serum, heart, brain, and skeletal muscle had significantly lower concentrations ( $P = .01$ , Table 1). Swainsonine clearance from serum had a half-life ( $T_{1/2}$ ) of  $18.5 \pm 2.5$  h. Similar rates were observed in skeletal muscle, heart, brain, and pancreas. Swainsonine in the liver, spleen, and kidney had significantly longer clearance rates, with  $T_{1/2}$  of over 50 h (Table 1). Although the liver, kidney, and spleen had relatively high swainsonine concentrations that were cleared much slower than other tissues, this did not alter serum swainsonine, which rapidly fell to below detectable concentrations. No swainsonine was detected in the serum 4 d after discontinuing locoweed dosing (Figure 1). Clearance from all tissues was consistent with first-order elimination rates because all produced linear regressions ( $r^2 > .6$ ).

Although not statistically significant, the brain tended to have higher swainsonine concentrations than the serum (405 ng/g of brain vs 350 ng/mL of serum). The brain also had slightly longer clearance, with a  $T_{1/2}$  of 32.2 h compared to the serum  $T_{1/2}$  of 18.5 h. No swainsonine was detected in any of the control tissues.

## Discussion

As seen in Figure 1, swainsonine intoxication for longer than 2 wk resulted in decreased weight gains. Similar findings have been reported in laboratory animals and naturally poisoned animals (Stegelmeier et al., 1995c; Galyean et al., 1996). It has been speculated that reduced animal performance and weight losses are due to the direct effects of locoweed. Swainsonine inhibits Golgi mannosidase II, resulting in abnormal intestinal and pancreatic exocrine enzyme glycosylation and excretion (Stegelmeier et al., 1995c). This could impair digestion, which is important in poisoning with other glycosidase inhibitors

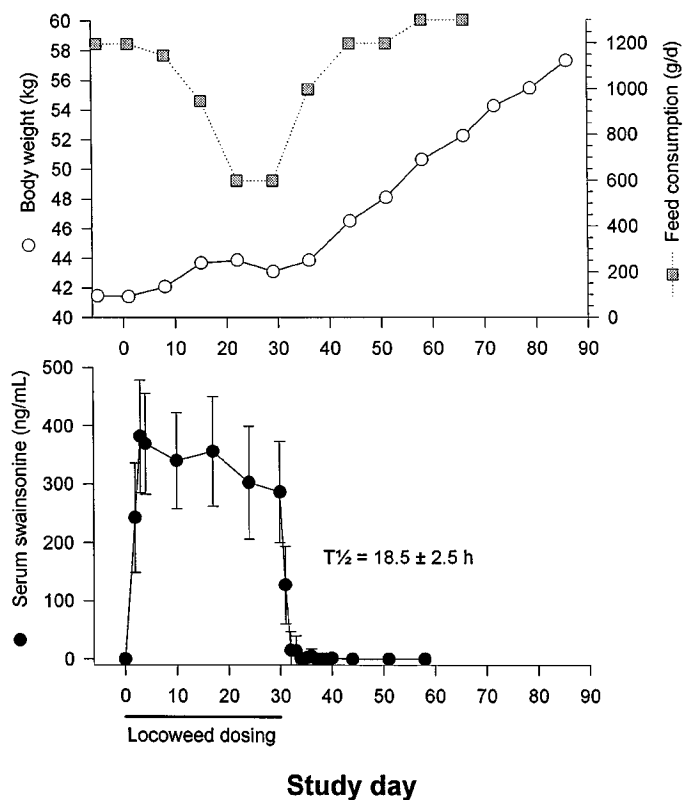


Figure 1. Weight gains, feed consumption, and serum swainsonine concentrations of wethers that were gavaged with locoweed to obtain a swainsonine dose of  $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 30 d.

that inhibit intestinal mucosal glycosidases (Pan et al., 1993). Neurologic anorexia probably also plays a role in locoweed poisoning as weight loss or the lack of weight gains in locoweed poisoning correspond with development of clinical depression and anorexia. Within several days after discontinuing locoweed dosing, all animals began to eat their complete ration, weight gains returned to normal, and no swainsonine was detected in the serum. This suggests that short locoweed exposures may be of little consequence and economic importance. However, little is known concerning the development and sequelae of repeated or intermittent poisoning. At some point, intoxication results in irreversible neurologic damage (Marsh, 1909; James and Van Kampen, 1971). Additional research is needed to define these limits of poisoning and better determine the prognosis for poisoned livestock.

Serum swainsonine concentrations from the wethers in this study were similar to those previously reported in sheep and cattle (Table 1). Additionally, the serum or whole blood clearance rates in this and the other studies are nearly identical to those in other livestock, having  $T_{1/2}$  of about 20 h. This clearance rate has limited the effectiveness of using serum swainsonine as a diagnostic indicator of locoweed poisoning. Within a few days after removal from

Table 1. Comparison of tissue swainsonine concentrations and clearance from sheep and cattle

Group/treatment	Serum	Muscle	Brain	Heart	Liver	Spleen	Kidney	Pancreas
Current study <sup>a</sup> (1 mg/kg)								
Swainsonine ng/g	350 ± 27 <sup>bb</sup>	237 ± 59 <sup>bb</sup>	408 ± 115 <sup>bb</sup>	188 ± 71 <sup>bb</sup>	2,659 ± 523 <sup>aa</sup>	2,660 ± 488 <sup>aa</sup>	1,599 ± 253 <sup>aa</sup>	2,317 ± 75 <sup>aa</sup>
Half-life, h	18.5 ± 2.5 <sup>aa</sup>	19.2 ± 3.9 <sup>aa</sup>	32.2 ± 1.7 <sup>cc</sup>	17.6 ± 2.4 <sup>aa</sup>	59.9 ± 7.1 <sup>bb</sup>	52.0 ± 5.0 <sup>bb</sup>	51.4 ± 7.5 <sup>bb</sup>	13.0 ± 1.0 <sup>aa</sup>
Wether study <sup>b</sup> (1 mg/kg)								
Swainsonine ng/g	577 ± 70	435 ± 91	469 ± 103	—	3,947 ± 457	—	2,373 ± 320	—
Half-life, h	—	—	—	—	—	—	—	—
Lactating Ewes <sup>c</sup> 1.5 mg/kg								
Swainsonine, ng/g	395 ± 23	—	—	—	—	—	—	—
Half-life, h	20.3 ± 7.2	—	—	—	—	—	—	—
Pregnant cattle <sup>d</sup> .8 mg/kg								
Swainsonine, ng/g	204 ± 42	—	—	—	—	—	—	—
Half-life, h	17.6 ± 2.4	—	—	—	—	—	—	—

<sup>a</sup>Wethers were dosed with locoweed to obtain swainsonine doses of  $1.0 \text{ mg/kg}$  for 30 d. Swainsonine was determined using a Jackbean  $\alpha$ -mannosidase inhibition assay (Stegelmeier et al., 1995a,b). Assay swainsonine standards were verified using capillary gas chromatography (Molyneux et al., 1989). Significantly different means ( $P < .05$ ) are indicated with small double letters.

<sup>b</sup>Wethers were fed pellets containing 10% ground *Astragalus lentiginosus* with alfalfa to obtain swainsonine doses of about  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 21 d. Swainsonine is reported from whole blood (Stegelmeier et al., 1995b).

<sup>c</sup>Lactating ewes were fed pellets containing 10% ground *Astragalus lentiginosus* with alfalfa to obtain swainsonine doses of about  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 12 d (Stegelmeier et al., 1995a).

<sup>d</sup>Pregnant cattle were gavaged with ground *Oxytropis sericea* to obtain doses of  $.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 30 d (Stegelmeier et al., 1995a).

locoweed exposure, obviously poisoned animals with prominent clinical signs can have very low or undetectable serum swainsonine concentrations. Interestingly, the neurologic signs of locoweed poisoning seem to become more prominent and severe during the first several days after animals discontinue eating locoweed. Anecdotal reports also suggest livestock removed from locoweed-infested ranges have exacerbated clinical signs. Such animals are more easily observed and more likely to be tested. More sensitive diagnostic methods with longer detection times are needed to identify swainsonine or its effects in such poisoned animals.

The sheep in this study accumulated significantly higher swainsonine concentrations in liver, kidney, and spleen than in serum. Swainsonine was also metabolized or cleared at much slower rates from these tissues (Table 1). In mice treated with a single intravenous injection of tritiated swainsonine, the liver, thymus, and kidney also had comparatively high swainsonine concentrations (Bowen et al., 1993). It was suggested that the rate-limiting step for swainsonine clearance in these mice was swainsonine movement from these tissues back into the blood. Interestingly, persistence of swainsonine in liver, kidney, and spleen in these wethers did not affect the serum swainsonine concentration or clearance. It may be that these tissues accumulate swainsonine or that clearance from these tissues occurs by some mechanism other than excretion via the blood stream. Liver and kidney have extensive sugar scavenging systems and are key players in sugar metabolism and conservation. Swainsonine, a mannose analog, may be actively accumulated via these same systems. The liver and kidney could also excrete swainsonine directly into the bile or urine, not allowing it reentry into circulation. Also, swainsonine from these tissues may be released at rates much slower than removal from the serum, resulting in undetectable serum concentrations. Under current conditions, the  $\alpha$ -mannosidase-inhibition assay has a detection limit of about 20 ng/mL; serum swainsonine concentrations below this level are not detected.

Bowen et al. (1993) in their pharmacokinetic study speculated that the relatively high swainsonine concentration present in the thymus may be an explanation for swainsonine's immunomodulatory effects. We did not analyze thymus, but we did find relatively high swainsonine concentrations in the spleen. Because the spleen is a lympho-reticular tissue, immunologic cells in the spleen may also accumulate swainsonine. This is supported by histologic findings; the macrophages and reticular cells in the spleen and other lymphoid tissue are some of the first cells to develop the characteristic vacuolation of locoweed poisoning (James and Van Kampen, 1971; Van Kampen and James, 1972).

Previous dose-response studies using rodents found that many tissues developed lesions in a threshold-

like fashion (Stegelmeier et al., 1995c). Certain swainsonine doses are required before lesions will develop in different tissues. After the tissue develops lesions, higher swainsonine doses do not result in more severe changes. It was hypothesized that this was due to inhibition of all the available mannosidase in those tissues. Current findings suggest that this threshold pattern is a direct result of the tissue swainsonine concentration. For example, the kidney accumulates relatively high swainsonine concentrations. As a result, the proximal convoluted tubules develop cytoplasmic vacuolation at relatively low systemic doses. Tissues with comparatively low swainsonine concentrations such as the brain require relatively high swainsonine doses to develop lesions. Additional studies are needed to more clearly delineate the swainsonine doses that result in neurotoxicity and determine whether intermittent poisoning or the duration of poisoning affects the progression or reversal of those changes.

The serum clearance calculated in this study was similar to those found in whole blood and serum of lactating ewes and pregnant cattle (Table 1). Swainsonine was cleared from skeletal muscle, heart, pancreas, and brain at similar rates, but the brain clearance rate was somewhat slower (Table 1). Although the liver, kidney, and spleen have significantly longer clearance times, they compose a relatively smaller portion of the body mass and represent a relatively small amount of swainsonine. The actual risk of swainsonine toxicosis from the ingestion of animal products is minimal. For all species that have been examined, locoweed must be consumed at doses above .2 mg swainsonine·kg<sup>-1</sup>·d<sup>-1</sup> for several days or weeks to develop even mild lesions (James and Van Kampen, 1971; Stegelmeier et al., 1995c). It would be nearly impossible to obtain similar doses from swainsonine-contaminated animal tissue. Nevertheless, to ensure quality of animal products, withdrawal times to allow for swainsonine clearance from all tissues is desirable. The time required to clear half the swainsonine from the liver is about 60 h, so animals need to be held off locoweed for about 25 d (10  $T_{1/2}$ ) to ensure swainsonine is cleared.

## Implications

Locoweed-poisoned sheep have swainsonine in many tissues. Tissue swainsonine concentrations seem correlated with the development of lesions in those tissues. Although swainsonine is rapidly cleared from the serum half-life ( $T_{1/2}$  = 18 h) after animals discontinue eating locoweed, it is cleared more slowly from the liver half-life ( $T_{1/2}$  = 60 h) and kidney half-life ( $T_{1/2}$  = 51 h). This suggests that a withdrawal time of about 25 d is necessary to allow swainsonine clearance from animals exposed to locoweed.



### Literature Cited

- Bowen, D., J. Adir, S. L. White, C. D. Bowen, K. Matsumoto, and K. Olden. 1993. A preliminary pharmacokinetic evaluation of the antimetastatic immunomodulator swainsonine: Clinical and toxic implications. *Anticancer Res.* 13:841.
- Galyean, M. L., M. H. Ralphs, M. N. Reif, J. D. Graham, and W. E. Braselton, Jr. 1996. Effects of previous grazing treatment and consumption of locoweeds on liver mineral concentrations in beef steers. *J. Anim. Sci.* 74:827.
- James, L. F., and K. R. Van Kampen. 1971. Acute and residual lesions of locoweeds poisoning in cattle and horses. *J. Am. Vet. Med. Assoc.* 158:614.
- Marsh, C. D. 1909. The locoweeds disease of the plains. USDA Bureau Animal Industry Bulletin No. 112. Washington, DC.
- Molyneux, R. J., L. F. James, K. E. Panter, and M. H. Ralphs. 1989. The occurrence and detection of swainsonine in locoweeds. In L. F. James, A. Elbein, R. J. Molyneux, and C. D. Warren (Ed.) *Swainsonine and Related Glycosidase Inhibitors*. pp 100-117. Iowa State University Press, Ames.
- Pan, Y. T., J. Ghidoni, and A. D. Elbein. 1993. The effects of castanospermine and swainsonine on the activity and synthesis of intestinal sucrase. *Arch. Biochem. Biophys.* 303:134.
- SAS. 1985. SAS User's Guide: Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.
- Stegelmeier, B. L., L. F. James, K. E. Panter, and R. J. Molyneux. 1995a. Serum swainsonine concentration and alpha-mannosidase activity in cattle and sheep ingesting *Oxytropis sericea* and *Astragalus lentiginosus* (locoweeds). *Am. J. Vet. Res.* 56:149.
- Stegelmeier, B. L., L. F. James, K. E. Panter, and R. J. Molyneux. 1995b. Tissue and serum swainsonine concentrations in sheep ingesting *Astragalus lentiginosus* (locoweeds). *Vet. Hum. Toxicol.* 37:336.
- Stegelmeier, B. L., R. J. Molyneux, A. D. Elbein, and L. F. James. 1995c. The comparative pathology of locoweeds, swainsonine, and castanospermine in rats. *Vet. Pathol.* 32:289.
- Van Kampen, K. R., and L. F. James. 1972. Sequential development of the lesions in locoweeds poisoning. *Clin. Toxicol.* 5:575.

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